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Two new species of *Nitocrella* (Crustacea, Copepoda, Harpacticoida) from groundwaters of northwestern Australia expand the geographic range of the genus in a global hotspot of subterranean biodiversity

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Abstract

In Australia, the Ameiridae is the most diverse harpacticoid family in groundwater, with 35 species hitherto reported. In this study, we describe two new species belonging to the "*vasconica*"-group of the ameirid genus *Nitocrella* based on specimens collected from groundwaters near mine sites in the Pilbara and Great Sandy Desert regions of northwestern Australia. *Nitocrella knotti* **sp. n.** can be distinguished from related taxa by having two setae on the antennal exopod, four armature elements on the distal endopodal segment of leg 2, four armature elements on the distal endopodal segment of leg 3, three armature elements on the distal endopodal segment of leg 4, and three setae on the basoendopodal lobe of leg 5. *Nitocrella karanovici* **sp. n.** differs from its congeners by having a short outer spine and long inner seta on the distal endopodal segment of leg 2, three armature elements on the distal endopodal segment of leg 3, and four setae on the basoendopodal lobe of leg 5 in the female. This study is of biogeographic interest in providing the first documentation of the genus *Nitocrella* from the Pilbara and Great Sandy Desert regions. Both new species of *Nitocrella* are recorded from restricted localities and appear to be short-range endemics, thus making them potentially vulnerable to environmental changes and threatening processes such as mining. The distribution range of *N. karanovici* **sp. n.** coincides with the centre of diversity of the Ethel Gorge aquifer stygobiont community, a globally significant hotspot which is listed as endangered.

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Keywords
Short-range endemic, stygofauna, compliance monitoring, mining

Introduction

The family Ameiridae (Copepoda) has successfully colonized and radiated in continental surface water and groundwater, with over 150 species reported from Australia, Asia, Europe, and North America (Boxshall and Halsey 2004, Boxshall and Defaye 2008). In Australia, the Ameiridae is the most diverse harpacticoid family in groundwater (Karanovic 2006, Karanovic and Hancock 2009). Presently 35 ameirid species have been reported from groundwater, mostly in Western Australia and a few in Queensland and South Australia (Table 1).

Groundwaters of (semi-) arid Western Australia are a globally significant hotspot for subterranean biodiversity (Humphreys 2008, Eberhard et al. 2009, Guzik et al. 2011). Most of this rich diversity occurs in two adjacent geographic regions, the Pilbara and the northern Yilgarn, both of which form parts of the Western Shield, a single emergent land mass since the Proterozoic (Fig. 1A). In both regions, progressive Quaternary climatic aridity is considered the major driver for groundwater colonization; however, there are some remarkable differences between the copepod and other stygofauna taxa of the Pilbara and northern Yilgarn, which have almost no genera in common and exhibit major differences in higher taxa (Karanovic 2006, Humphreys 2008, 2012). An explanation for this great biogeographic disjunction remains elusive.

The majority of ameirid species known from groundwater in Australia were collected at, or adjacent to, proposed mine sites which have been surveyed for stygofauna as part of the mine project environmental impact assessment, or, as part of the ongoing environmental compliance monitoring at established mine sites. Other ameirids were collected from pastoral wells and groundwater boreholes during the course of surveys by government departments, museums, and universities. Most of the described ameirids are recorded from single localities and appear to be short-range endemic species (sensu Harvey 2002, Harvey et al. 2011), and this makes them potentially vulnerable to environmental changes and threatening processes such as mining and groundwater abstraction. In some cases this has led to conflict between the competing interests of biodiversity conservation and resources development (see Karanovic et al. 2013). In this study, we describe two new ameirid species belonging to the genus *Nitocrella* Chappuis, 1923 based on specimens collected from groundwaters near two mine sites in the Pilbara and Great Sandy Desert regions of northwestern Western Australia.

Methods

The net-haul method (see Eberhard et al. 2005, 2009) was used to collect samples of stygofauna in July 2008 from one borehole located 48 km from the Telfer Mine in

Table 1. Harpacticoid copepods of the family Ameiridae reported from subterranean waters of Australia (in alphabetical order).

Species	State*	Reference
Abnitocrella eberhardi Karanovic, 2006	WA	Karanovic (2006)
Abnitocrella halsei Karanovic, 2006	WA	Karanovic (2006)
Antistygonitocrella pardalotos Karanovic, Eberhard, Perina & Callan, 2013	WA	Karanovic et al. (2013)
Archinitocrella newmanensis Karanovic, 2006	WA	Karanovic (2006)
Biameiropsis barrowensis Karanovic, 2006	WA	Karanovic (2006)
Gordanitocrella trajani Karanovic & Hancock, 2009	WA	Karanovic and Hancock (2009)
Haifameira pori Karanovic, 2004	WA	Karanovic (2004)
Hirtaleptomesochra bispinosa Karanovic, 2004	WA	Karanovic (2004)
Inermipes humphreysi Lee & Huys, 2002	WA	Lee and Huys (2002)
Kimberleynitocrella billhumphreysi Karanovic & Hancock, 2009	WA	Karanovic and Hancock (2009)
Lucionitocrella yalleenensis Karanovic & Hancock, 2009	WA	Karanovic and Hancock (2009)
Megastygonitocrella bispinosa (Karanovic, 2006)	WA	Karanovic (2006), Karanovic and Hancock (2009)
Megastygonitocrella dec Karanovic & Hancock, 2009	WA	Karanovic and Hancock (2009)
Megastygonitocrella ecowisei Karanovic & Hancock, 2009	WA	Karanovic and Hancock (2009)
Megastygonitocrella embe Karanovic, Eberhard, Perina & Callan, 2013	WA	Karanovic et al. (2013)
Megastygonitocrella kryptos Karanovic & Hancock, 2009	QLD	Karanovic and Hancock (2009)
Megastygonitocrella pagusregalis Karanovic & Hancock, 2009	QLD	Karanovic and Hancock (2009)
Megastygonitocrella trispinosa (Karanovic, 2006)	WA	Karanovic (2006), Karanovic and Hancock (2009)
Megastygonitocrella unispinosa (Karanovic, 2006)	WA	Karanovic (2006), Karanovic and Hancock (2009)
Nitocrella absentia Karanovic, 2004	WA	Karanovic (2004)
Nitocrella obesa Karanovic, 2004	WA	Karanovic (2004)
Nitocrella trajani Karanovic, 2004	WA	Karanovic (2004)
Nitocrellopsis halsei Karanovic, 2010	WA	Karanovic (2010)
Nitocrellopsis operculata Karanovic, 2010	WA	Karanovic (2010)
Nitocrellopsis pinderi Karanovic, 2010	WA	Karanovic (2010)
Nitokra esbe Karanovic, Eberhard, Cooper & Guzik, 2014	WA	Karanovic et al. (2014)
Nitokra humphreysi Karanovic & Pesce, 2002	WA	Karanovic and Pesce (2002)
Nitokra lacustris (Schmankevitsch, 1895)	SA	Zeidler (1989)
Nitokra lacustris pacifica Yeatman, 1983	WA	Karanovic (2004), Tang and Knott (2009)
Nitokra yeelirrie Karanovic, Eberhard, Cooper & Guzik, 2014	WA	Karanovic et al. (2014)
Novanitocrella aboriginesi Karanovic, 2004	WA	Karanovic (2004)
Paranitocrella bastiani Tang & Knott, 2009	WA	Tang and Knott (2009)
Parapseudoleptomesochra karamani Karanovic, 2004	WA	Karanovic (2004)
Parapseudoleptomesochra rouchi Karanovic, 2004	WA	Karanovic (2004)
Parapseudoleptomesochra tureei Karanovic, 2006	WA	Karanovic (2006)

*WA = Western Australia; QLD = Queensland; SA = South Australia

the Great Sandy Desert region of Western Australia, and annually, from 2009–2012, from a total of eight boreholes in the Ethel Gorge aquifer adjacent to two mine pits, namely Orebody 23 and Orebody 25, 15 km NE of Newman in the Pilbara region of Western Australia (Fig. 1). Each net-haul sample was transferred to a labelled 33 ml

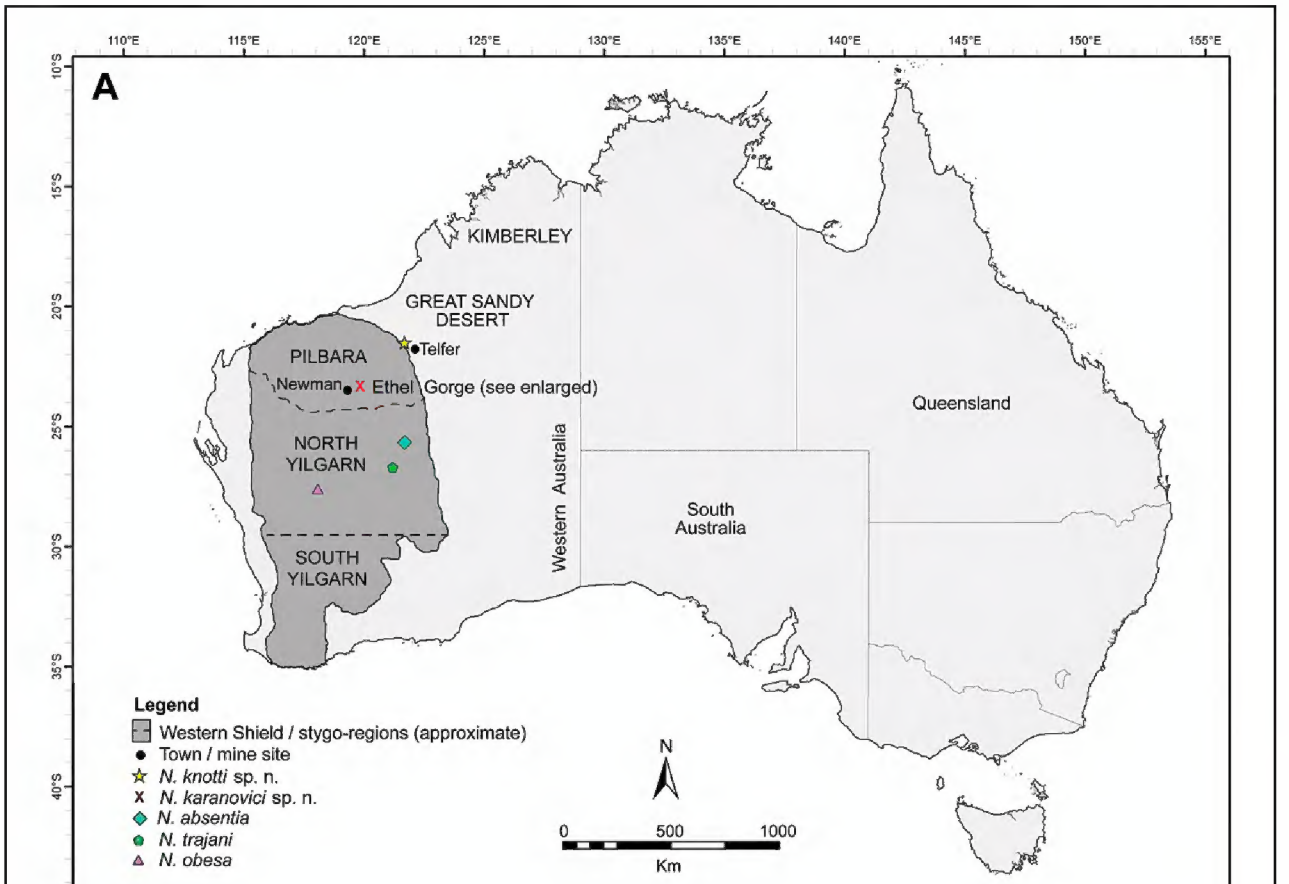

Figure 1. A Map showing the species of *Nitocrella* reported from Western Australia **B** Enlarged map of the Ethel Gorge area showing sampled boreholes and collection sites for *Nitocrella karanovici* sp. n. in relation to surface drainage and mine pits.

vial and preserved in absolute alcohol for possible future molecular studies (although molecular studies were not required for this taxonomic description of *Nitocrella* species which were well defined on the basis of morphology). Samples were transported to the laboratory where copepods were sorted from debris and other stygofauna using a dissecting microscope.

Copepod specimens selected for taxonomic study were soaked in lactophenol prior to examination using a Leica M205C dissecting microscope and Leica MD2500 compound microscope equipped with differential interference contrast. Selected specimens were measured using an ocular micrometer, dissected, and examined using the wooden slide procedure of Humes and Gooding (1964). Selected whole specimens and dissected appendages were also drawn with the aid of a drawing tube. Morphological terminology follows Huys and Boxshall (1991). Type material was deposited in the Western Australian Museum (WAM), Kewdale, Australia.

Results

Order Harpacticoida Sars, 1903
Family Ameiridae Boeck, 1865
Genus *Nitocrella* Chappuis, 1923

***Nitocrella knotti* sp. n.**
http://zoobank.org/A2B3DDF8-47A5-4435-B405-5AF902BF18BA
Figs 2–4

Type locality. Borehole HB54/4.1 (= Borehole HB405) (21°28'45"S; 121°46'45"E) on Telfer Road, 48 km NW of Telfer mine site and 350 km SE of Port Hedland, Western Australia, July 2008, J. Mifsud leg.

Type material. Holotype female (WAM C51830) in absolute alcohol, 1 female paratype (WAM C51831) dissected and mounted on a slide, and 4 female paratypes (WAM C51832) in absolute alcohol.

Description. *Female.* Body (Fig. 2A) subcylindrical, 610–635 μm (mean 620 μm; n = 3) long (measured from tip of rostrum to posterior margin of caudal rami) and 148–155 μm (mean 153 μm; n = 3) wide (at posterolateral margin of cephalothorax). Prosome composed of cephalothorax and 3 free pedigerous somites; tergite of first two pedigerous somites each with elliptical integumental window. Urosome comprised of fifth pedigerous somite, genital double-somite, and 3 free abdominal somites. Fifth pedigerous somite with short, dorsolateral row of spinules and numerous short rows of minute denticles (not drawn) and minute surface pits (not drawn) on dorsal and ventral surfaces. Components of genital double-somite (Fig. 2A, B) partially fused dorsally but completely fused ventrally, ornamented with short, anterolateral row of spinules on ventral surface, minute surface pits and numerous short rows of minute denticles on dorsal and ventral surfaces (only minute denticles on ventral surface are

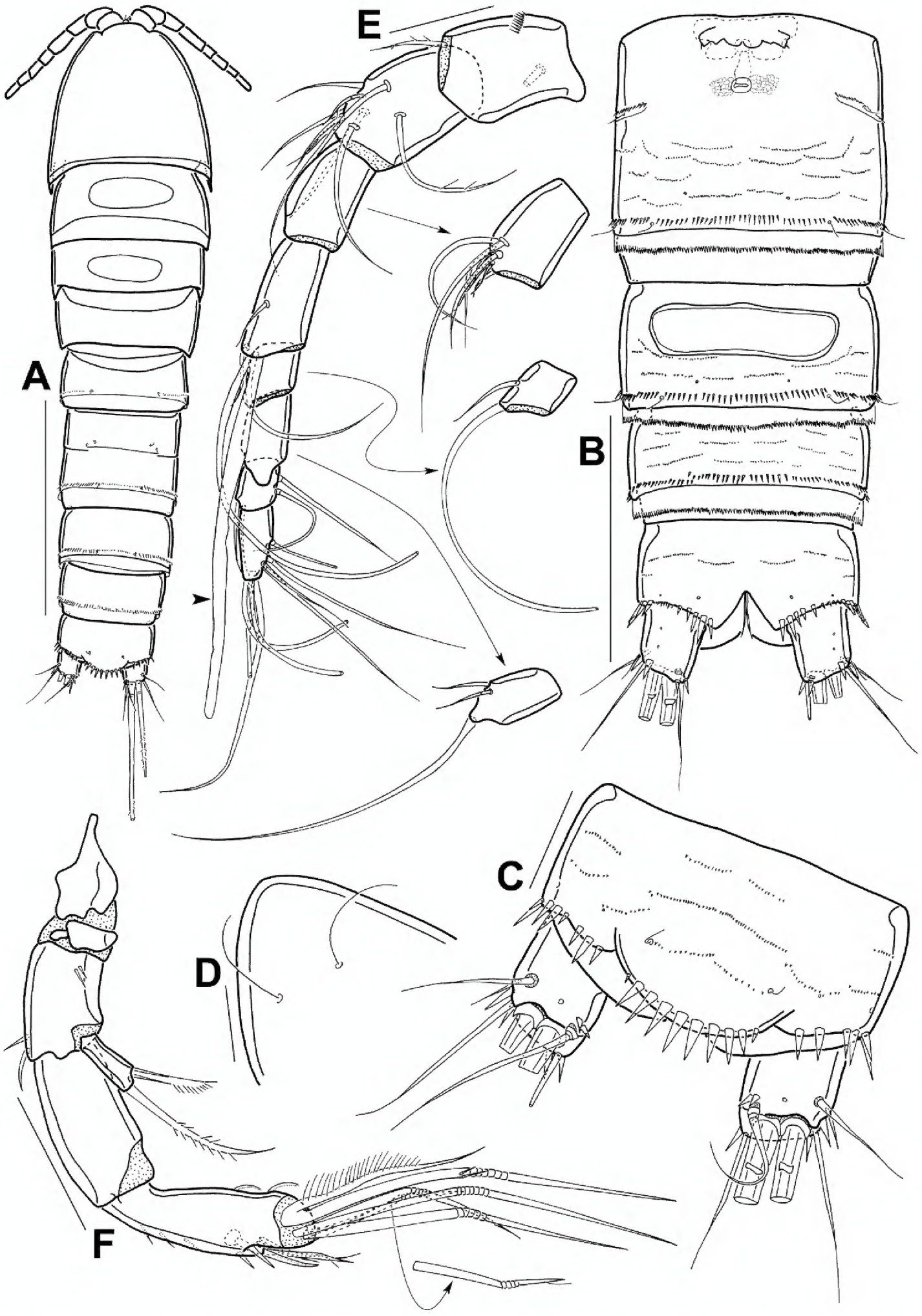

Figure 2. *Nitocrella knotti* sp. n., adult female: **A** habitus, dorsal **B** urosomites 2–5 and caudal rami, ventral **C** anal somite and caudal rami, dorsal **D** rostrum, dorsal **E** right antennule with segments 3, 5 and 6 shown separately and aesthetasc indicated by arrowhead, ventral **F** left antenna with one apical element shown separately, anterior. Scale bars: **A** 200 μm; **B** 100 μm; **C, E, F** 25 μm; **D** 2 μm.

shown), and row of large spinules and serrated hyaline frill encircling posterior margin; genital field with large median copulatory pore, chitinized copulatory duct leading anteriorly to pair of bilobate seminal receptacles, and median genital pore covered by operculiform leg 6. First free abdominal somite with large integumental window on ventral surface, minute surface pits and numerous short rows of minute denticles on dorsal and ventral surfaces (only minute denticles on ventral surface are shown), and row of large spinules and serrated hyaline frill ringing posterior margin. Second free abdominal somite similar to preceding somite, but without integumental window. Anal somite (Fig. 2B, C) with minute surface pits (not drawn) and numerous short rows of minute denticles on dorsal and ventral surfaces and row of large spinules along posterior border and along posterior margin of anal operculum.

Caudal ramus (Fig. 2B, C) short, about 1.25 times longer than wide, bears minute surface pits (not drawn) and 7 setae. Insertion point of setae III, VI and VII flanked by spinules. Setae IV and V spinulate, with proximal breaking planes; other setae naked. Seta VII basally tri-articulate.

Rostrum (Fig. 2D) subtrianglar, not demarcated at base, with 2 dorsal sensilla.

Antennule (Fig. 2E) 8-segmented, with armature as follows: 1, 9, 8, 4 + ae, 2, 3, 4, and 8. Segment 1 proximally with additional spinular row and large tubular pore. Two (of 4) and 4 (of 8) setae basally biarticulate on segments 7 and 8, respectively. Two (of 4) anterodistal setae on segment 8 fused at base.

Antenna (Fig. 2F), comprising coxa, basis, exopod, and 2-segmented endopod. Coxa naked and unarmed. Basis with 2 small proximal spinules and 2 large inner distal spinules. Exopod 1-segmented, cylindrical, and armed with 2 pinnate setae. Proximal endopodal segment naked and unarmed. Distal endopodal segment as long as basis and proximal endopodal segment combined; ornamented with 2 distolateral hyaline frills, 3 proximomedial spinules, and 2 distomedial spinules; armed with 2 spines (1 spine with minute spinules along inner margin; other with subapical flagellum) plus 2 naked setae along inner subdistal margin and 1 pilose and 5 geniculate setae along apical margin (lateralmost geniculate seta with 2 spinules at mid-point and fused basally with pilose seta; shortest geniculate seta with subapical flagellum).

Labrum (Fig. 3A) subtriangular, with denticles along apical margin and large distolateral denticles plus 2 patches of minute denticles on posterior face.

Mandible (Fig. 3B) composed of coxa and 2-segmented palp. Coxa with inner subapical process, numerous unicuspidate teeth along distal margin, and unilaterally denticulate seta on inner distal angle. Proximal segment of palp unarmed, but furnished with 2 proximomedial spinules, 1 medial process, and row of apical spinules; distal segment armed with 5 apical naked setae.

Maxillule (Fig. 3C) composed of praecoxa and 3-segmented palp. Praecoxal arthrite bears proximal crescentic row of spinules, 2 chitinized naked setae on inner subapical margin, 2 long naked setae on anterior surface, and 8 apical elements (3 highly chitinized, of which 2 each furnished with minute apical teeth; 1 unipinnate; 1 with bristled tip; 3 naked). Coxal endite elongated, with subapical row of spinules and 1 geniculate and 2 naked setae at distal end. Basis as long as coxa, bears 5 apical

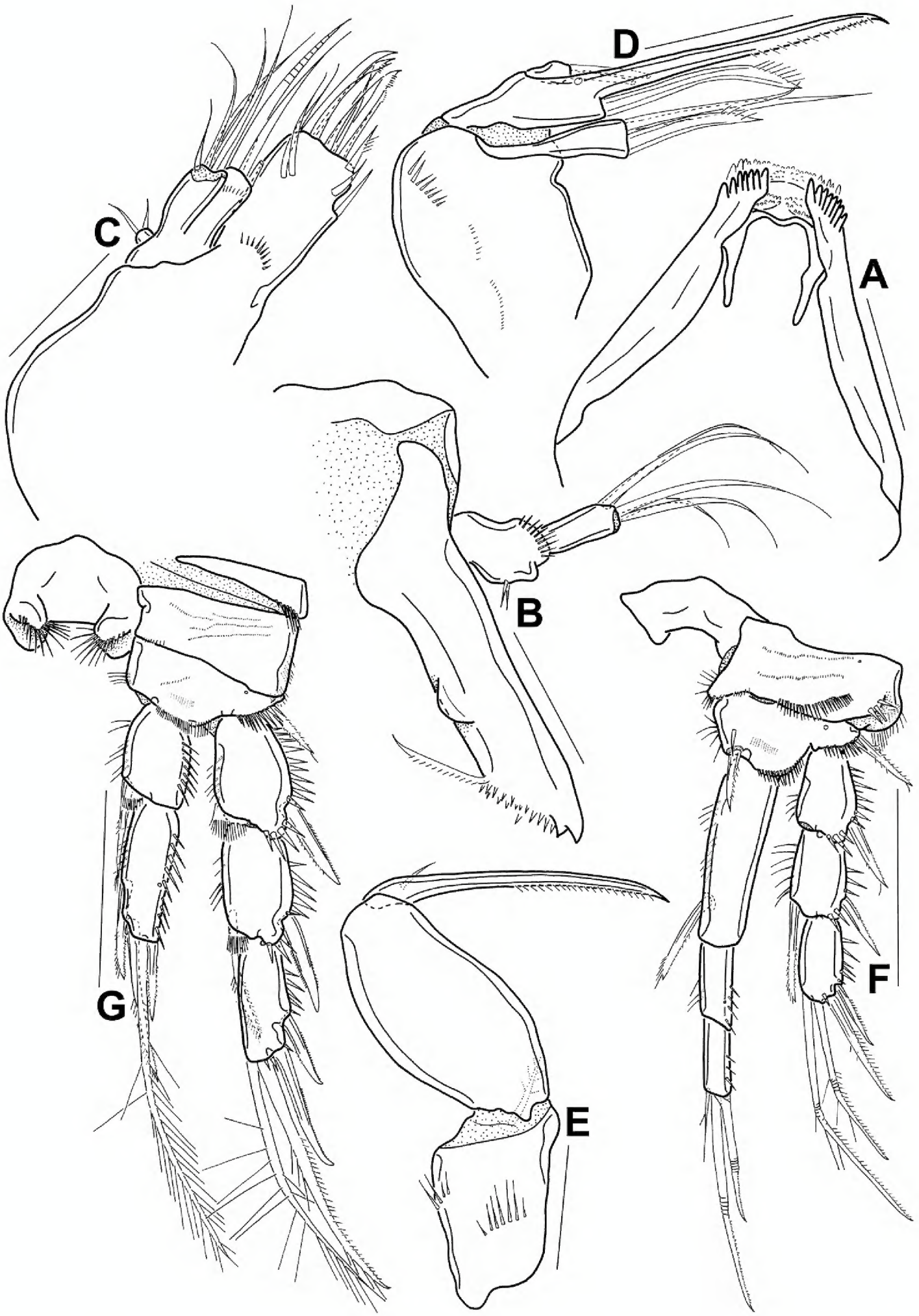

Figure 3. *Nitocrella knotti* sp. n., adult female: **A** labrum, posterior **B** left mandible, anterior **C** left maxillule, anterior **D** left maxilla, anterior **E** right maxilliped, posterior **F** left leg 1, anterior **G** left leg 2, anterior. Scale bars: **A, B, C, D, E** 20 μm; **F, G** 50 μm.

naked setae. Endopod 1-segmented, vestigial, armed with 2 short naked setae. Exopod absent.

Maxilla (Fig. 3D) 3-segmented, composed of syncoxa, allobasis, and 1-segmented endopod. Syncoxa large, with 3 longitudinal rows of spinules on anterior surface and 1 pectinate and 2 naked apical setae on distal endite. Allobasis drawn out into long claw furnished with spinules along distal half of inner margin and bears 1 pectinate seta. Endopod 1-segmented, armed with 2 long apical setae.

Maxilliped (Fig. 3E) 3-segmented, comprising syncoxa, basis, and 1-segmented endopod. Syncoxa stout, with 2 rows of long spinules and 1 distal pinnate seta. Basis naked and unarmed, about 1.5 times as long as syncoxa. Endopod drawn out into long claw, with 1 proximal naked seta and denticles along distal half of inner margin.

Legs 1–4 biramous (Figs 3F, G, 4A, B); leg 1 with trimerous rami; legs 2–4 with trimerous exopod and bimerous endopod. Armature on rami of legs 1 to 4 as follows (Roman numerals = spines; Arabic numerals = setae):

	Coxa	**Basis**	**Exopod**	**Endopod**
Leg 1	0-0	I-I	I-0; I-I; II,2,0	0-I; 0-0; 0,3,0
Leg 2	0-0	I-0	I-0; I-I; II,2,0	0-I; 0,I+1,II
Leg 3	0-0	1-0	I-0; I-I; II,2,0	0-I; 0,I+1,1+I
Leg 4	0-0	1-0	I-0; I-I; II,2,2	0-I; 0,I+1,I

Leg 1 (Fig. 3F) intercoxal sclerite naked and about twice as wide as long. Coxa with 2 rows of minute spinules and 1 row of large spinules on anterior surface; outer margin with 1 row of large spinules and 2 rows of fine spinules; inner distal corner with row of fine spinules. Basis with row of large spinules at insertion of each ramus and row of fine spinules along inner margin and on posterior surface; 1 additional large spinule present near base of inner spine; both spines with subapical flagellum. Outer spine on proximal exopodal segment with subapical flagellum. First two exopodal segments with large spinules along outer margin and on outer distal corner, as well as fine spinules along inner margin; distal segment with large spinules along outer margin and 1 spinule on apical margin. Both setae on terminal exopodal segment geniculate. Proximal endopodal segment long, extending almost to mid-point of distal exopodal segment, with fine spinules along inner margin; middle segment with fine spinules along outer and inner margins; distal segment with fine spinules along outer margin. Two (of 3) setae on distal endopodal segment geniculate. Both rami with minute surface pits (not drawn).

Leg 2 (Fig. 3G) intercoxal sclerite posteriorly bilobate, with row of spinules on each lobe. Coxa with 4 rows of minute spinules on anterior surface; outer margin with 2 rows of fine spinules; inner distal corner with row of minute spinules. Basis ornamented as in leg 1, except lacks large spinule near inner margin and with additional row of minute spinules on anterior surface. Exopod ornamented as in leg 1, except with additional spinulated frill on inner distal corner of proximal and middle segments. First two exopodal segments protruded on outer distal corner. Proximal endopodal segment with spinules along outer and inner margins and short spinulated frill on

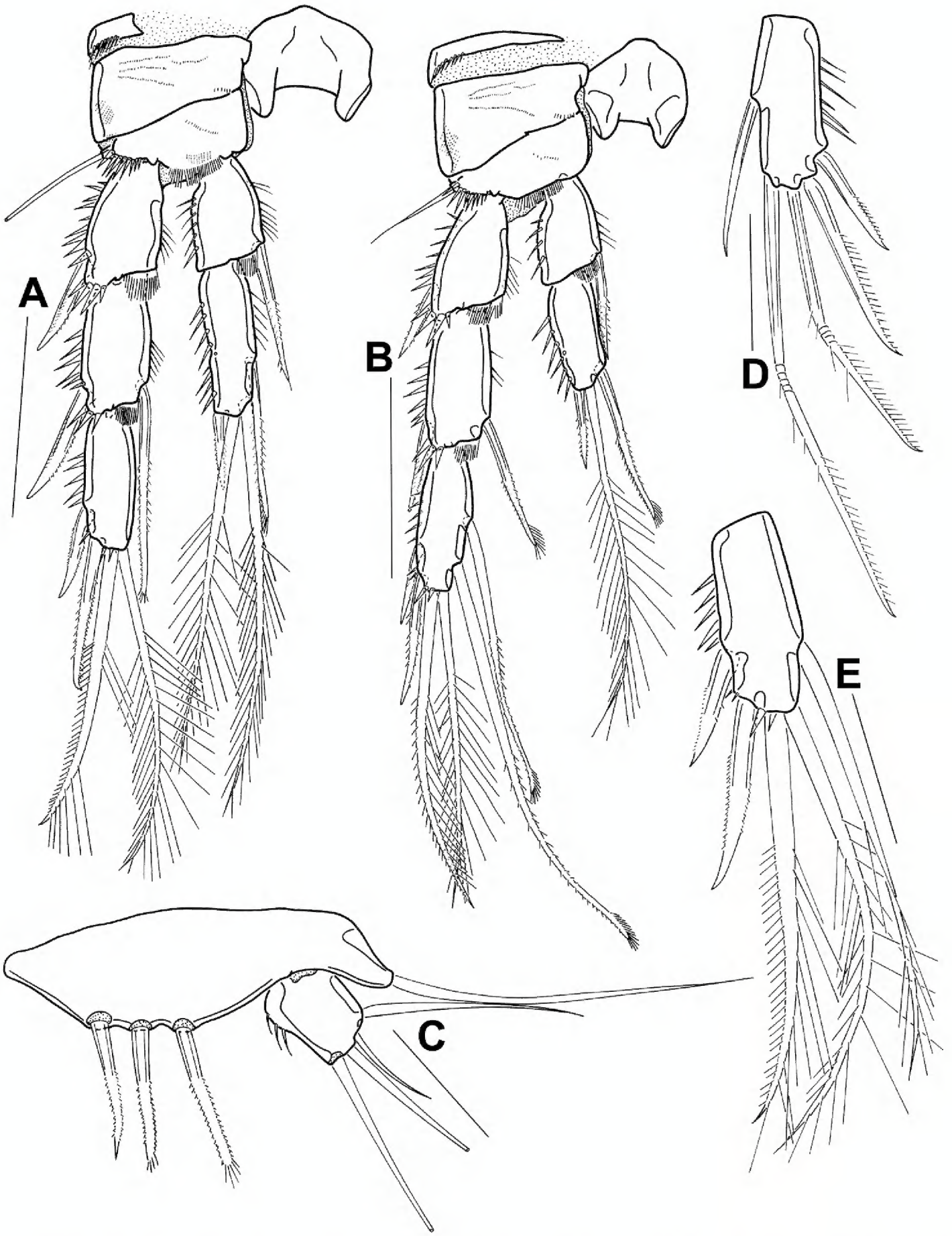

Figure 4. *Nitocrella knotti* sp. n., adult female: **A** right leg 3, anterior **B** right leg 4, anterior **C** left leg 5, ventral **D** terminal exopodal segment of left leg 1, anterior **E** terminal exopodal segment of right leg 2, anterior. Scale bars: **A, B** 50 μm; **C, D** 20 μm; **E** 25 μm.

inner distal corner. Distal endopodal segment about 1.5 times longer than proximal endopodal segment and furnished with spinules along outer and inner margins. Both rami with minute surface pits (not drawn) as in leg 1.

Leg 3 (Fig. 4A) similar to leg 2, except with naked intercoxal sclerite, outer seta (instead of spine) on basis, longer inner spine on middle exopodal segment, and inner distal seta (instead of spine) and longer inner proximal spine on distal endopodal segment.

Leg 4 (Fig. 4B) similar to leg 3, except with smaller intercoxal sclerite, row of spinules absent on posterior surface of basis, less protruded outer distal corner on first two exopodal segments, shorter inner spine on middle exopodal segment, 2 more elements on distal exopodal segment, shorter distal endopodal segment, and only 3 elements on distal endopodal segment.

Leg 5 (Fig. 4C) biramous. Basoendopod with long outer basal seta and 3 spinulated setae on endopodal lobe. Exopod 1-segmented, slightly longer than wide, with few spinules along inner margin and 4 unequal naked setae.

Leg 6 (Fig. 2B) represented by simple operculum covering genital pore, armed with 1 minute naked seta on outer distal corners.

Male. Unknown.

Variability. One paratype with 5 elements on terminal exopodal segment of left leg 1 (Fig. 4D). Another paratype with 5 elements on terminal exopodal segment of right leg 2 (Fig. 4E).

Etymology. This species is named in honour of the late Professor Brenton Knott (The University of Western Australia) who made significant contributions to research on groundwater fauna in Western Australia.

Differential diagnosis. Among the three groups of *Nitocrella* proposed by Petkovski (1976), i.e. "*chappuisi*", "*hirta*", and "*vasconica*", *Nitocrella knotti* sp. n. belongs to the "*vasconica*"-group as it also possesses the characteristic six armature elements on the distal exopodal segment of leg 4. With the addition of *N. knotti* sp. n. (including the second new species described below), this group currently contains 21 species reported from Eurasia, the Caribbean, and Australia (Table 2). *Nitocrella knotti* sp. n. shares with *N. afghanica* Štěrba, 1973, *N. jankowskajae* Borutzky, 1972, *N. kirgizica* Borutzky, 1972, *N. monchenkoi* Borutzky, 1972, *N. obesa* Karanovic, 2004, and *N. trajani* Karanovic, 2004 an armature formula of I-0; I-I; II,2,0 on the exopod and 0-I; 0-0; 0,3,0 on the endopod of leg 1, II,2,0 on the distal exopodal segment of legs 2 and 3, and 0-I on the proximal endopodal segment of legs 2–4. However, *N. knotti* sp. n. can be easily distinguished from those taxa by having four armature elements (instead of two for *N. jankowskaja*, or three for *N. afghanica*, *N. kirgizica*, *N. monchenkoi*, *N. obesa*, and *N. trajani*) on the distal endopodal segment of leg 2. *Nitocrella knotti* sp. n. differs further from the Australian *N. obesa* and *N. trajani* by having the genital and first abdominal somites fused ventrally (rather than completely separate), two setae (rather than three) on the antennal exopod, and three setae (rather than four) on the basoendopodal lobe of leg 5, among others; and from the Central Asian *N. afghanica*, *N. jankowskajae*, *N. kirgizica*, and *N. monchenkoi* by having four armature elements (instead of two) on the distal endopodal segment of leg 3 and three armature elements (instead of one for *N. afghanica*, or two for *N. jankowskaja*, *N. kirgizica*, and *N. monchenkoi*) on the distal endopodal segment of leg 4.

Table 2. Species of *Nitocrella* belonging to the "*vasconica*"-group (in alphabetical order).

Species	Locality	Reference
N. absentia Karanovic, 2004	Australia	Karanovic (2004)
N. afghanica Štěrba, 1973	Afghanistan	Štěrba (1973)
N. beatricis Cottarelli & Bruno, 1993	Sardinia (Italy); Corsica (France)	Cottarelli and Bruno (1993)
N. caraioni Petkovski, 1976	Cuba	Petkovski (1976)
N. dussarti Chappuis & Rouch, 1959	Pyrenees (France)	Chappuis and Rouch (1959)
N. gracilis Chappuis, 1955	Pyrenees (France)	Chappuis (1955), Rouch (1964)
N. knotti sp. n.	Australia	Present study
N. jankowskajae Borutzky, 1972	Kirgiziya (= Kyrgyzstan)	Borutzky (1972)
N. karanovici sp. n.	Australia	Present study
N. kirgizica Borutzky, 1972	Kirgiziya (= Kyrgyzstan)	Borutzky (1972)
N. mara Löffler, 1959	Iran	Löffler (1959)
N. monchenkoi Borutzky, 1972	Uzbekistan	Borutzky (1972)
N. motasi Petkovski, 1976	Cuba	Petkovski (1976)
N. nana Štěrba, 1973	Afghanistan	Štěrba (1973)
N. obesa Karanovic, 2004	Australia	Karanovic (2004)
N. paceae Pesce, 1980	Iran	Pesce (1980)
N. stetinai Štěrba, 1973	Afghanistan	Štěrba (1973)
N. trajani Karanovic, 2004	Australia	Karanovic (2004)
N. unispinosa Shen & Tai, 1973	China	Shen and Tai (1973)
N. vasconica Chappuis, 1937	Spain	Chappuis (1937)
N. yokotai Miura, 1962	Japan	Miura (1962)

***Nitocrella karanovici* sp. n.**
http://zoobank.org/BF208E60-B0C2-4AF1-9857-04716000206E
Figs 5–9

Type locality. Borehole UNK02 (23°15'00"S; 119°53'41"E), Ethel Gorge aquifer, approximately 15 km ENE of Newman (Fig. 1), Western Australia, 8 February 2011, P. Bell and S. Catomore leg.

Type material. Holotype female (WAM C51837) in absolute alcohol, allotype male (WAM C51838) in absolute alcohol, 4 paratype females and 2 paratype males (WAM C51839–C51844) dissected and mounted on one slide each, and 26 paratype females, 28 paratype males and 1 copepodid (WAM C51845) in absolute alcohol.

Other material examined. All material collected from boreholes in the Ethel Gorge aquifer, approximately 15 km ENE of Newman, Western Australia (Fig. 1). 3 females and 1 male (WAM C51833) in absolute alcohol, borehole EEX917 (23°19'47"S; 119°52'13"E), 8 February 2012, S. Catomore and N. Coen leg.; 7 females (WAM C51834) in absolute alcohol, borehole EEX917 (23°19'47"S; 119°52'13"E), 12 April 2012, P. Bell and S. Catomore leg.; 1 male (WAM C51835) in absolute alcohol, borehole P13S (23°18'56"S; 119°50'58"E), 21 November 2009, P. Bell and G. Perina leg.; 1 female (WAM C51836) in absolute alcohol, borehole T399 (23°17'03"S;

119°52'07"E), 5 November 2010, P. Bell and S. Catomore leg.; 13 females and 3 males (WAM C51846) in absolute alcohol, borehole UNK02 (23°15'00"S; 119°53'41"E), 9 February 2011, P. Bell and S. Catomore leg.; 2 females (WAM C51847) in absolute alcohol, borehole W105 (23°19'37"S; 119°51'50"E), 9 February 2011, P. Bell and S. Catomore leg.; 7 females (WAM C51848) in absolute alcohol, borehole W105 (23°19'37"S; 119°51'50"E), 13 April 2012, P. Bell and S. Catomore leg.; 3 females (WAM C51849) in absolute alcohol, borehole W105 (23°19'37"S; 119°51'50"E), 10 February 2012, S. Catomore and N. Coen leg.; 2 females (WAM C51850) in absolute alcohol, borehole W116 (23°14'47"S; 119°54'25"E), 8 February 2011, P. Bell and S. Catomore leg.; 3 females (WAM C51851) in absolute alcohol, borehole W116 (23°14'47"S; 119°54'25"E), 22 November 2009, P. Bell and G. Perina leg.; 5 females (WAM C51852) in absolute alcohol, borehole W116 (23°14'47"S; 119°54'25"E), 21 April 2010, P. Bell and G. Perina leg.; 8 females and 1 male (WAM C51853) in absolute alcohol, borehole W116 (23°14'47"S; 119°54'25"E), 8 February 2012, S. Catomore and N. Coen leg.; 6 females and 3 males (WAM C51854) in absolute alcohol, borehole W116 (23°14'47"S; 119°54'25"E), 11 April 2012, P. Bell and S. Catomore leg.; 1 female (WAM C51855) in absolute alcohol, borehole W79D (23°19'42"S; 119°50'39"E), 22 November 2009, P. Bell and G. Perina leg.; 3 females (WAM C51856) in absolute alcohol, borehole W79D (23°19'42"S; 119°50'39"E), 12 April 2012, P. Bell and S. Catomore leg.; 2 males (WAM C51857) in absolute alcohol, borehole WP126NRE (23°15'01"S; 119°53'42"E), 21 November 2009, P. Bell and G. Perina leg.

Description. *Female*. Body (Fig. 5A) cylindrical, 450–495 µm (mean 471 µm; n = 6) long (measured from tip of rostrum to posterior margin of caudal rami) and 95–105 µm (mean 103 µm; n = 6) wide (at first free pedigerous somite). Prosome composed of cephalothorax and 3 free pedigerous somites. Urosome comprised of fifth pedigerous somite, genital double-somite, and 3 free abdominal somites. Components of genital double-somite (Fig. 5A, B, C) not fused dorsally but completely fused ventrally, with elliptical integumental window laterally, row of small spinules immediately posterior to each integumental window, and row of large spinules and frill of minute spinules encircling posterior margin; genital field with large median copulatory pore, chitinized copulatory duct leading anteriorly to pair of lobate seminal receptacles, and median genital pore covered by operculiform leg 6. First free abdominal somite with anteroventral pair of oval integumental windows and row of unequal spinules and frill of minute spinules ringing posterior border. Second free abdominal somite with row of subequal spinules and frill of minute spinules encircling posterior edge. Anal somite (Fig. 5B, D, E) with anterior and posterior row of spinules on ventral surface, several rows of spinules on lateral surface, and spinules along posterior margin of anal operculum.

Caudal ramus (Fig. 5B, D, E) about 1.5 times longer than wide, bearing 7 setae. Spinules present at insertion point of setae III, VI and VII. Setae IV and V spinulate, with proximal breaking planes; other setae naked. Seta VII basally tri-articulate.

Rostrum (Fig. 5F) about 1.25 times longer than wide, not demarcated at base, with rounded apex and 2 dorsal sensilla.

Antennule (Fig. 6A) similar to that of *N. knotti* sp. n.

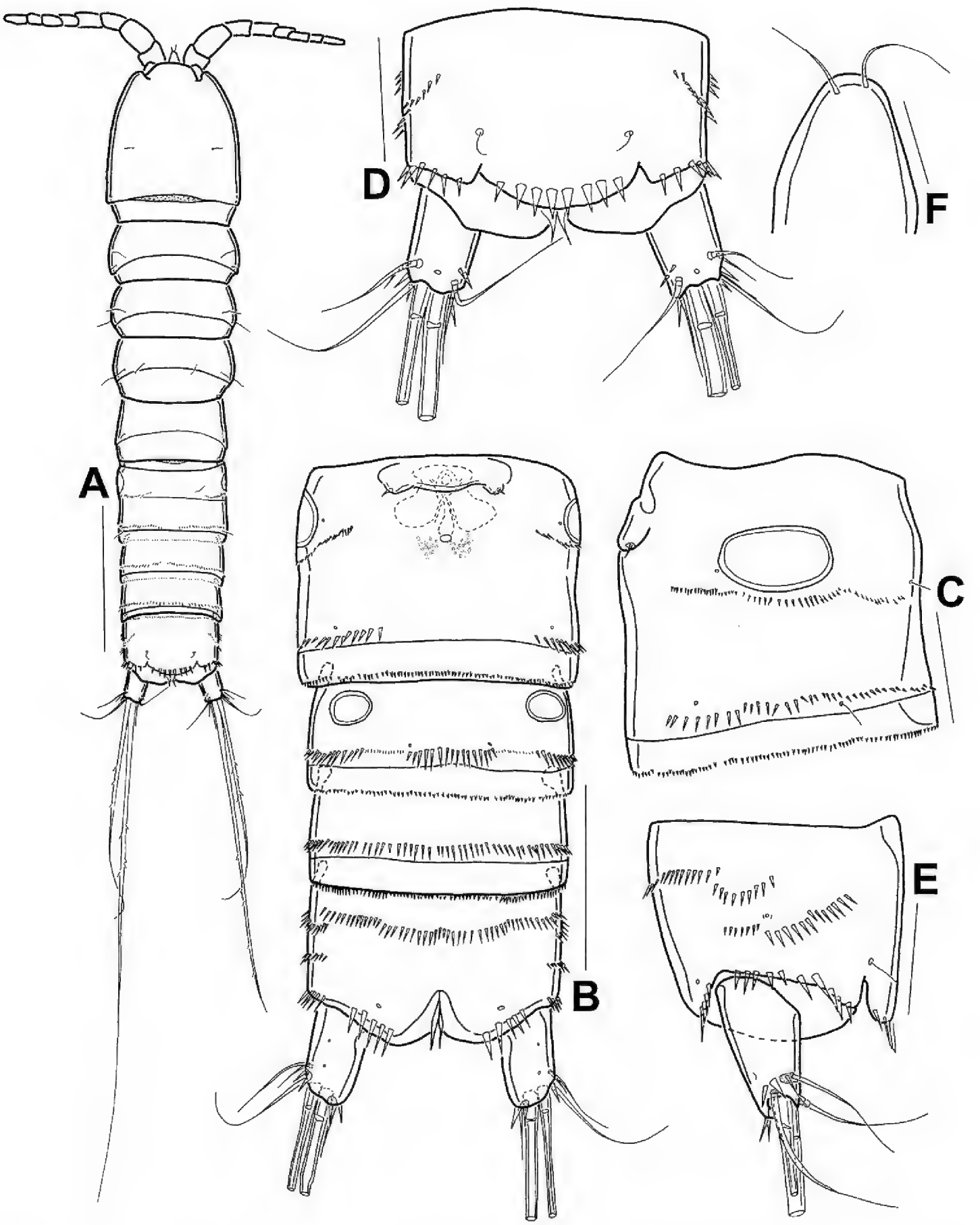

Figure 5. *Nitocrella karanovici* sp. n., adult female: **A** habitus, dorsal **B** urosomites 2–5 and caudal rami, ventral **C** genital double-somite, lateral **D** anal somite and caudal rami, dorsal **E** anal somite and left caudal ramus, lateral **F** rostrum, dorsal. Scale bars: **A** 100 μm; **B** 50 μm **C, D, E** 25 μm; **F** 5 μm.

Antenna (Fig. 6B), comprising coxa, basis, 1-segmented exopod, and 2-segmented endopod. Coxa naked and unarmed. Basis with 2 long spinules on inner margin and short row of minute spinules on anterior surface. Exopod armed with 3 pinnate

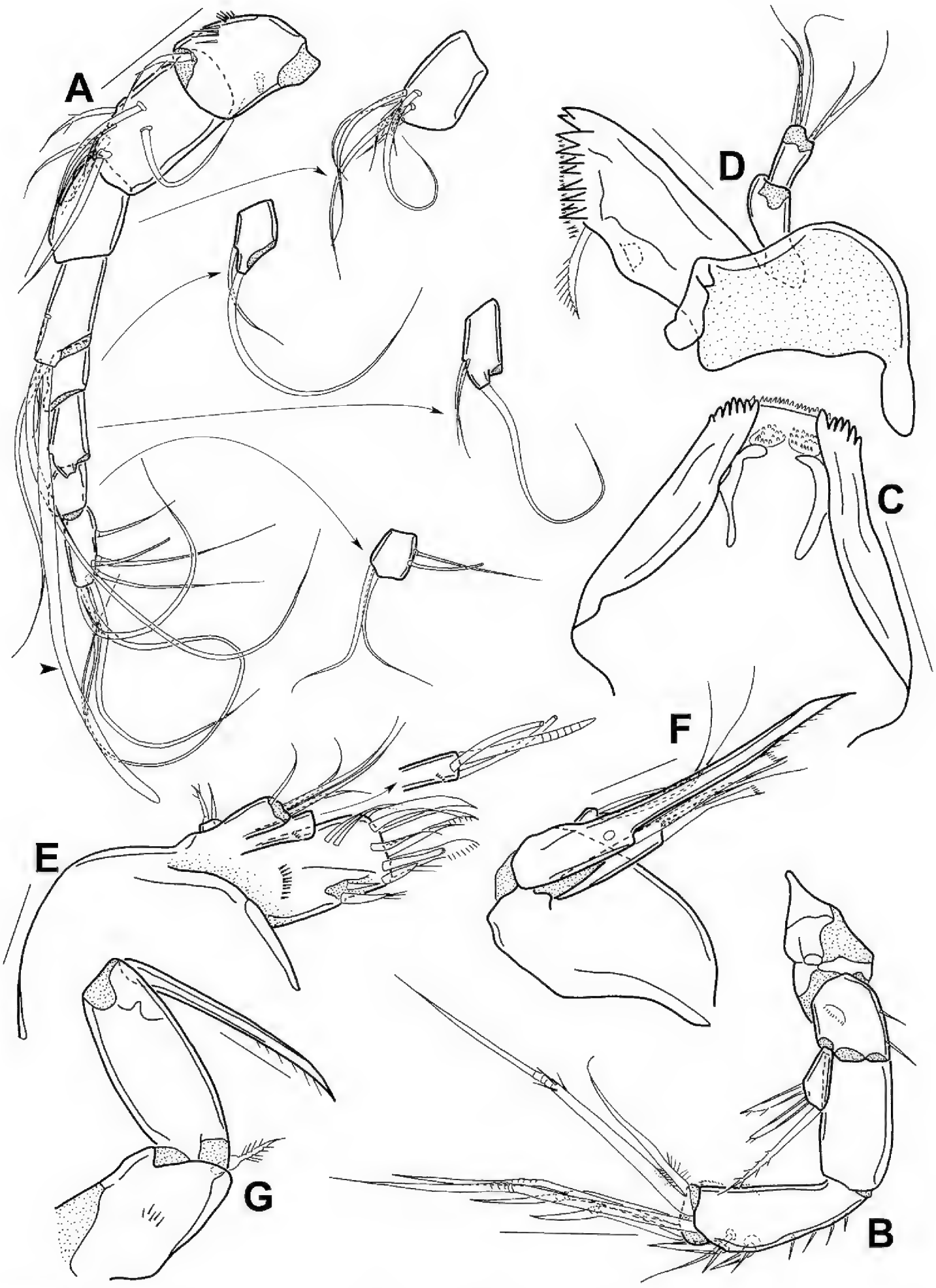

Figure 6. *Nitocrella karanovici* sp. n., adult female: **A** right antennule with segments 3, 5, 6 and 7 shown separately and aesthetasc indicated by arrowhead, ventral **B** right antenna, anterior **C** labrum, posterior **D** left mandible, posterior **E** left maxillule, anterior **F** left maxilla, anterior **G** right maxilliped, posterior. Scale bars: **A** 50 μm; **B, C** 20 μm; **D, E, F, G** 10 μm.

setae (2 highly chitinized). Proximal endopodal segment naked and unarmed. Distal endopodal segment slightly longer than proximal endopodal segment; ornamented with row of spinules on proximal half of inner margin, 2 distolateral hyaline frills, and 2 distomedial spinules; armed distomedially with 2 naked unequal spines plus 1 long naked seta and apically with 1 pilose and 5 geniculate setae (lateralmost geniculate seta with 2 spinules at mid-point and fused basally with pilose seta).

Labrum (Fig. 6C) as in *N. knotti* sp. n., except shorter and with smaller denticles along distal margin.

Mandible (Fig. 6D) similar to that of *N. knotti* sp. n.

Maxillule (Fig. 6E) similar to that of *N. knotti* sp. n.

Maxilla (Fig. 6F) as in *N. knotti* sp. n., except with naked syncoxa.

Maxilliped (Fig. 6G) similar to that of *N. knotti* sp. n., except with only 1 row of spinules on syncoxa and no proximal seta on endopod.

Legs 1–4 biramous (Fig. 7A, B, C, D); leg 1 with trimerous rami; legs 2–4 with trimerous exopod and bimerous endopod. Armature on rami of legs 1 to 4 as follows (Roman numerals = spines; Arabic numerals = setae):

	Coxa	**Basis**	**Exopod**	**Endopod**
Leg 1	0-0	I-I	I-0; I-I; III,2,0	0-I; 0-0; 0,I+2,0
Leg 2	0-0	I-0	I-0; I-I; II,2,1	0-I; 0,I+1,0
Leg 3	0-0	1-0	I-0; I-I; II,2,1	0-I; 0,I+1,I
Leg 4	0-0	1-0	I-0; I-I; II,2,1+I	0-I; 0,I+1,0

Leg 1 (Fig. 7A) intercoxal sclerite naked and concave on posterior margin. Coxa with 1 row of spinules on anterior surface and another row of spinules on posterolateral surface. Basis with row of long spinules at insertion of each ramus and 3 additional large spinules proximal to inner spine; both spines with subapical flagellum. Exopodal segments with large spinules along outer margin and on outer distal corner; middle segment also with fine spinules along inner margin. Endopodal segments with large spinules on outer margin and fine spinules along inner margin. Both setae on terminal exopodal segment and 1 of 3 setae on distal endopodal segment geniculate.

Leg 2 (Fig. 7B) intercoxal sclerite naked and posteriorly bilobate. Coxa with 1 row of minute spinules on posterolateral surface. Basis with row of spinules at insertion of each ramus and several fine spinules (only 1 depicted) on inner margin; outer spine with subapical flagellum. Exopod ornamented as in leg 1, except with additional spinulated frill on inner distal corner of proximal and middle segments. First two exopodal segments protruded on outer distal corner. Both endopodal segments with spinules along outer margin.

Leg 3 (Fig. 7C) similar to leg 2, except with outer seta (instead of spine) on basis and 3 elements on distal endopodal segment.

Leg 4 (Fig. 7D) similar to leg 3, except with much smaller spinules at insertion of endopod, 6 elements on distal exopodal segment (of which inner distal seta is longer

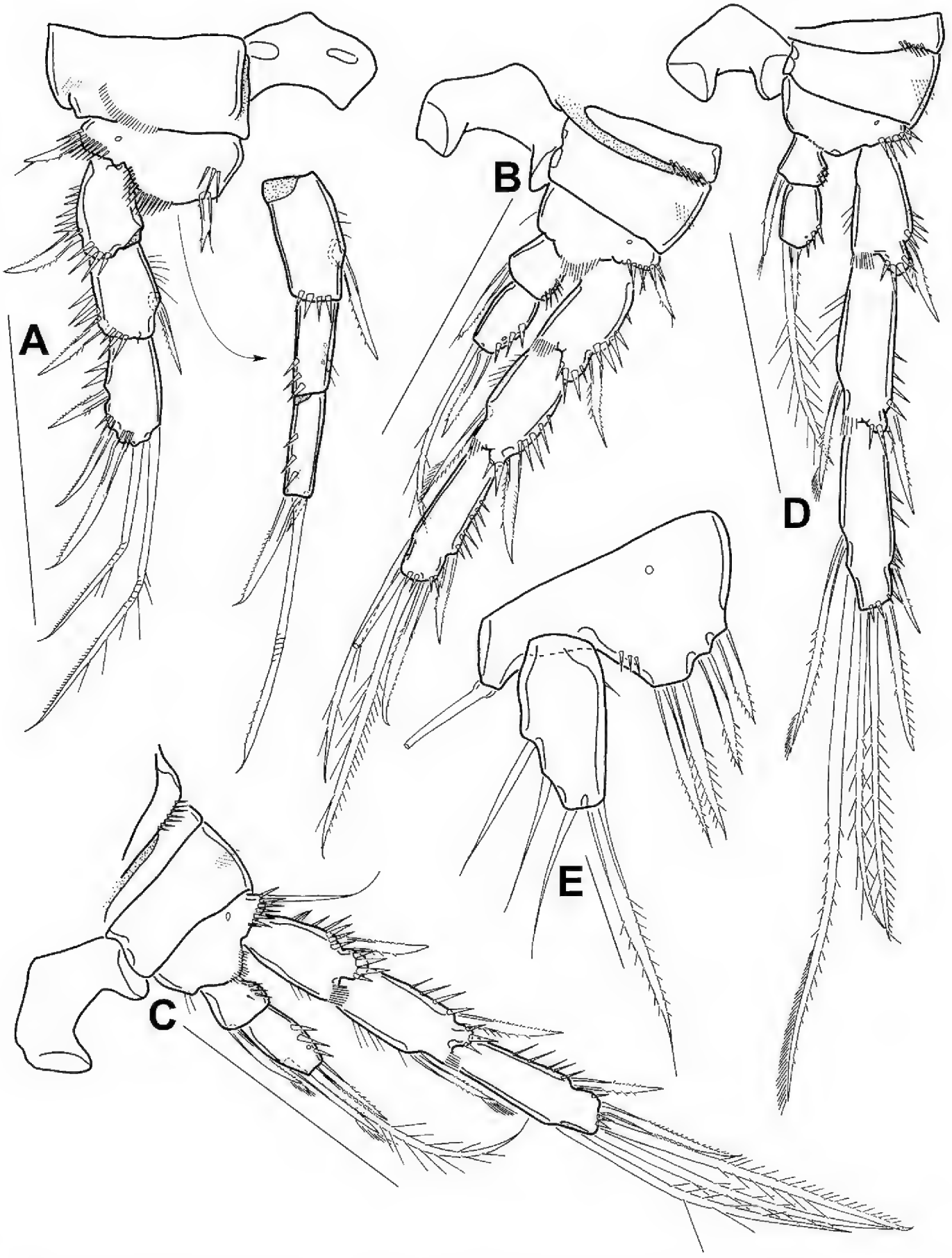

Figure 7. *Nitocrella karanovici* sp. n., adult female: **A** right leg 1 with endopod disarticulated from basis, anterior **B** left leg 2, anterior **C** left leg 3, anterior **D** left leg 4 (note: outer seta on basis is broken off), anterior **E** right leg 5, ventral. Scale bars: **A, B, C, D** 50 μm; **E** 10 μm.

and ornamented with tightly packed spinules on inner margin of apex), and 2 elements on distal endopodal segment.

Leg 5 (Fig. 7E) biramous. Basoendopod with long outer basal seta plus median pore, short row of spinules laterally and 4 distal spinulated setae on endopodal lobe.

Exopod 1-segmented, about twice as long as wide, with spinules along inner margin and 4 setae (3 naked; 1 spinulated).

Leg 6 (Fig. 5B) represented by genital operculum covering genital pore, and armed with 1 minute naked seta on distolateral borders.

Male. Body length (measured from tip of rostrum to posterior margin of caudal rami) 400–440 μm (mean 417 μm; n = 7); body width 90–95 μm (mean 91 μm; n = 7). Prosome composed of cephalothorax and 3 free pedigerous somites. Urosome comprised of fifth pedigerous somite, genital somite and 4 free postgenital somites. Genital somite (Fig. 8A) wider than long, with elliptical integumental window laterally and row of small spinules immediately posterior to each integumental window. First postgenital somite (Fig. 8A) with ventral pair of oval integumental windows, posterior row of large spinules, and frill of minute spinules along posterior border. Second and third postgenital somites (Fig. 8A) posteriorly with row of spinules and frill of minute spinules. Anal somite (Fig. 8A) ornamented as in female. Caudal ramus (Fig. 8A) about 2 times as long as wide; armed and ornamented as in female.

Antennule (Fig. 8B) 10-segmented, haplocerate, with geniculation between segments 7 and 8. Armature as follows: 1, 10, 8, 2, 7 + ae, 2, 3, 4, 4, and 8. Short spinulate seta(e) with flagellate tip present on segments 5–7. Aesthetasc and adjacent apical seta on segment 5 basally fused forming acrothek. One (of 3) and 3 (of 4) elements on segments 7 and 8, respectively, modified as digitate spines. Two apical setae on segment 10 basally fused.

Inner spine on basis of leg 1 (Fig. 8C) modified as is typical for members of Ameiridae.

Leg 5 (Fig. 8D) biramous, with basoendopods fused medially. Basoendopod with outer basal seta and median pore and 2 apical spinulated elements on endopodal lobe. Exopod 1-segmented, about 1.3 times as long as wide, with 5 setae (3 naked; 2 spinulate).

Leg 6 (Fig. 8A) asymmetrical, with right side modified as operculum and left side basally fused to somite; each side armed with 2 unequal distolateral setae.

Variability. One paratype female with discontinuous row of spinules along posteroventral margin of anal somite (Fig. 5B), but row is continuous in other paratype specimens. One dissected paratype female and 1 intact paratype male with 4 elements on terminal exopodal segment of leg 1 (Fig. 8E). One dissected and 1 intact paratype males with 3 elements on distal endopodal segment of leg 2 (Fig. 9C). One dissected paratype female with 4 elements on terminal exopodal segment of leg 3 (Fig. 8F). One dissected paratype female and 1 intact paratype male with 2 elements on distal endopodal segment of leg 3 (Fig. 8G). One dissected and 3 intact paratype females with longer inner distal spine on distal endopodal segment of leg 3 (Fig. 8H). One dissected and 3 intact paratype females plus 3 intact paratype males with 3 elements on distal endopodal segment of leg 4 (Fig. 8I). One intact paratype female with 1 element on distal endopodal segment of leg 4 (Fig. 8J). Two dissected and 1 intact paratype females with 3 (Fig. 9A) or 2 elements (Fig. 9B) on basoendopod of leg 5. Five intact paratype males with 1 (Fig. 9D) or no elements

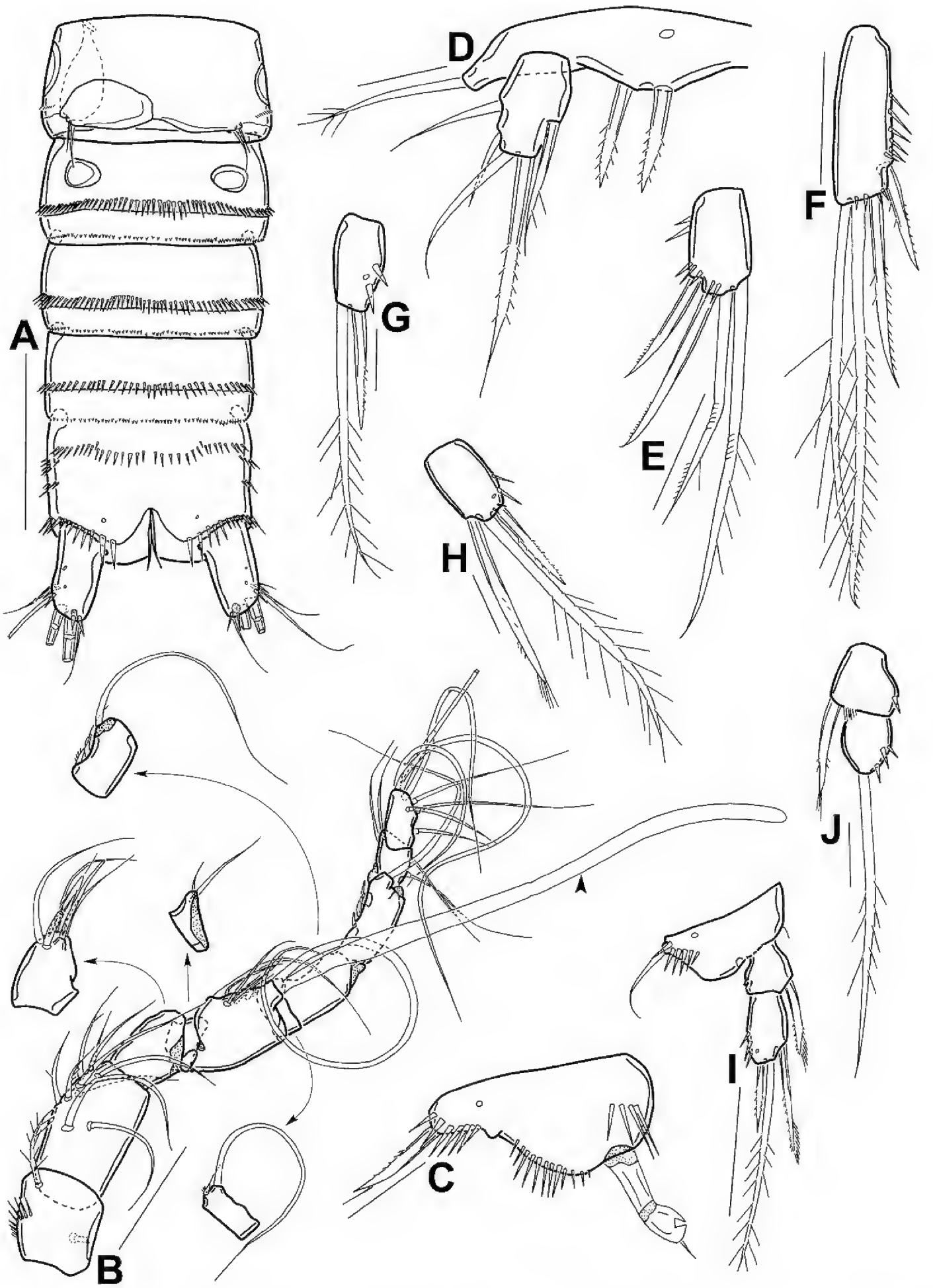

Figure 8. *Nitocrella karanovici* sp. n., adult male (**A, B, C, D**) and adult female (**E, F, G, H, I, J**): **A** urosomites 2–6 and caudal rami, ventral **B** left antennule with segments 3, 4, 6 and 7 shown separately and aesthetasc indicated by arrowhead, ventral **C** right leg 1 basis, anterior **D** right leg 5, ventral **E** terminal exopodal segment of right leg 1, anterior **F** terminal exopodal segment of left leg 3, anterior **G** terminal endopodal segment of left leg 3, anterior **H** same, anterior **I** basis and endopod of right leg 4, anterior **J** endopod of left leg 4, anterior. Scale bars: **A** 50 μm; **B, I** 25 μm; **C, D, G, H, J** 10 μm; **E, F** 20 μm.

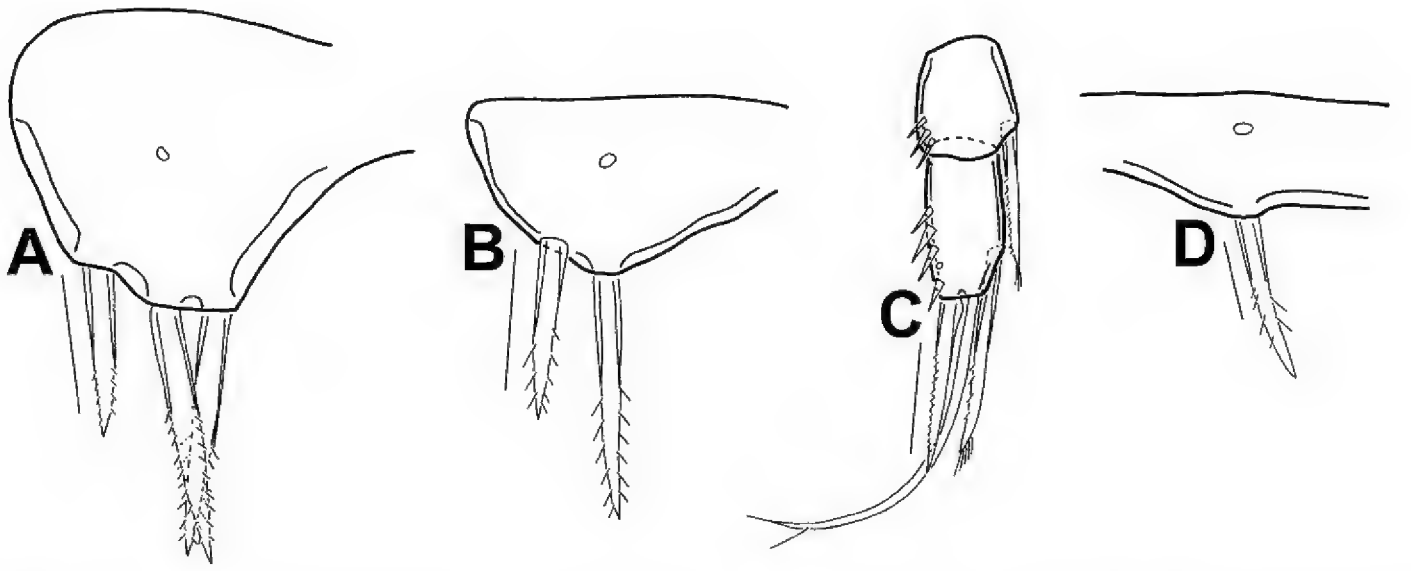

Figure 9. *Nitocrella karanovici* sp. n., adult female (**A, B**) and adult male (**C, D**): **A** basoendopod of left leg 5, ventral **B** same, ventral **C** endopod of right leg 2, anterior **D** basoendopod of left leg 5, ventral. Scale bars: **A, B, C** 10 μm; **D** 5 μm.

(not drawn) on basoendopod of leg 5. One intact paratype male with 3 setae on leg 6 (not drawn).

Etymology. This species is named for Dr. Tomislav Karanovic, in recognition of his extensive taxonomic research on subterranean copepods of Australia.

Differential diagnosis. *Nitocrella karanovici* sp. n. also belongs to the "*vasconica*"-group as it possesses the distinctive six armature elements on the distal exopodal segment of leg 4. Of the other 20 species in this group, *N. karanovici* sp. n. shares five armature elements on the distal exopodal segment of leg 1 with only *N. dussarti* Chappuis & Rouch, 1959 and *N. gracilis* Chappuis, 1955. *Nitocrella karanovici* sp. n. can be easily distinguished from *N. dussarti* by having three armature elements (instead of four) on the distal endopodal segment of leg 3 and four setae (instead of three) on both the exopod and basoendopodal lobe of leg 5 in the female, and from *N. gracilis* by having a short outer spine and long inner seta (rather than two subequal setae) on the distal endopodal segment of leg 2, two spines and one seta (instead of one spine and two setae) on the distal endopodal segment of leg 3, and four setae (instead of 3) on the basoendopodal lobe of leg 5 in the female.

Discussion

This study is of biogeographic interest in providing the first documentation of the genus *Nitocrella* from the Pilbara and Great Sandy Desert regions of northwestern Australia. Previously in Australia, *Nitocrella* was known only from three species in the northern Yilgarn region (Karanovic 2004) (Fig. 1A). Karanovic (2006) recognized that the subterranean copepod fauna is strikingly dissimilar, particularly at the genus level, between the neighboring Pilbara and Yilgarn regions (of which the Murchison region forms a part). Prior to this study, only *Schizopera* G. O. Sars, 1905 and *Nitocrellopsis* Galassi,

De Laurentiis & Dole-Olivier, 1999 were known from both regions. Indeed, the Pilbara region has stronger copepod faunal connections to the Kimberley region in Western Australia, and to northern Queensland, than to the northern Yilgarn region (Karanovic 2006, Karanovic and Hancock 2009). This study brings the number of copepod genera now known to be shared between the Pilbara and northern Yilgarn to three, although the addition of this third copepod genus makes little difference to further understanding of the perplexing taxonomic dissimilarities between these two adjacent stygo-regions.

Karanovic (2010) noted that the species of *Nitocrellopsis* reported from the two regions are only remotely related to each other. The same trend also appears to be the case for the species of *Nitocrella*. For example, although all five species of *Nitocrella* collected from Western Australia belong to the "*vasconica*"-group, they differ from each other in many respects (see Table 3). *Nitocrella karanovici* sp. n. and *N. knotti* sp. n. both have the genital and first abdominal somites fused ventrally, but they differ in the number and position of the integumental windows, the armature of the antennal exopod, and the armature of legs 1 to 5. *Nitocrella obesa* and *N. trajani* share the same number of armature elements on legs 1, 2 and 4, but they differ in the body ornamentation and armature of legs 3 and 5. *Nitocrella absentia* Karanovic, 2004 and *N. karanovici* sp. n. have the same

Table 3. Comparison of morphological characters between female *Nitocrella* species reported from Western Australia.

Character	*N. absentia*	*N. obesa*	*N. trajani*	*N. knotti* sp. n.	*N. karanovici* sp. n.
Genital and first abdominal somites fused ventrally	No	No	No	Yes	Yes
Integumental window on body	Absent	Absent	Absent	1 present on 2nd and 3rd prosomites and on 3rd urosomite	1 pair present on 2nd and 3rd urosomites
Spinules along posterior margin of anal operculum	Present	Absent	Present	Present	Present
No. of setae on antennal exopod	3	3	3	2	3
No. of armature elements on distal exopodal segment of leg 1	4	4	4	4	5
Spinules on distolateral lobes of intercoxal sclerite of leg 2	Absent	Present	Absent	Present	Absent
No. of armature elements on distal exopodal segment of legs 2 and 3	5	4	4	4	5
No. of armature elements on distal endopodal segment of leg 2	4	3	3	4	2
No. of armature elements on distal endopodal segment of leg 3	4	3	4	4	3
Outer spine on middle exopodal segment of leg 4	Absent	Present	Present	Present	Present
No. of armature elements on distal endopodal segment of leg 4	4	3	3	3	2
No. of armature elements on basoendopodal lobe of leg 5	4	1	4	3	4

armature on the exopod of legs 2 and 3 and on the basoendopod of leg 5, but they differ in the number and ornamentation of the urosomites and armature of leg 4.

The collection records for both *Nitocrella karanovici* sp. n. and *N. knotti* sp. n. strongly suggest that each species is a short-range endemic (SRE), with their recorded distribution ranges being much less than the SRE thresholds of 10 000 km^2 nominated by Harvey (2002), or 1 000 km^2 recommended by Eberhard et al. (2009) for Pilbara stygofauna. Both collection localities have been subjected to multiple monitoring surveys over some 15 years. Out of 30 boreholes monitored in eight surveys at Telfer, *Nitocrella* sp. has only been recorded from two closely adjacent boreholes (HB405 and HB406) (Bennelongia 2010). Other stygofaunal taxa collected from borehole HB405 included copepods, ostracods, and paramelitid amphipods. The aquifer type, groundwater depth, and physicochemistry in the Telfer boreholes were not available to this study.

Out of more than 40 boreholes monitored over numerous surveys at Ethel Gorge, *N. karanovici* sp. n. has only been recorded from eight boreholes that span a linear range of less than 14 km (Figure 1B). Twenty-three other stygofauna species were collected from the eight boreholes sampled during the four surveys (2009 to 2012) in Ethel Gorge which detected *N. karanovici* sp. n., including: harpacticoid (3 species) and cyclopoid (4) copepods, candonid (9) and limnocytherid (1) ostracods, paramelitid amphipods (3), tainisopid isopods (1), parabathynellid syncarids (1), and naidid (1) and phreodrilid (2) oligochaetes (Subterranean Ecology 2014). In three boreholes (EEX917, T399, W105), *N. karanovici* sp. n. occurred sympatrically with another ameirid, *Archinitocrella newmanensis* Karanovic, 2006. Both these taxa share a similar habitus and while *A. newmanensis* was often very abundant in samples *N. karanovici* sp. n. was represented by only a few individuals. For these reasons *N. karanovici* sp. n. may have missed detection during earlier monitoring surveys.

Eighty-four species of stygofauna have been recorded from the Ethel Gorge aquifer and adjacent groundwaters in the Newman area, thus ranking it as one of the richest localized groundwater fauna assemblages in Australia, and indeed globally (Subterranean Ecology 2013, 2014, Halse et al. 2014). At least 45 species, including *N. karanovici* sp. n., are considered to be stygobionts (obligate groundwater species) because they possess morphological specializations to subterranean life and are not known from surface waters. Around 40 of the stygobiont species have to date only been recorded from the Ethel Gorge aquifer or adjacent groundwaters in the Newman area (Subterranean Ecology 2013). The Ethel Gorge aquifer stygobiont community is therefore a local hotspot within a regional hotspot. The centre of species richness and abundance for this stygobiont community is concentrated in the shallow alluvial and calcrete aquifer around Ethel Gorge where the Fortescue River flows through the Ophthalmia Range. It is effectively a subsurface gorge where the bedrock shallows and the watertable lies generally less than ten metres below ground level (BHP undated). The distribution range of *N. karanovici* sp. n. more or less coincides with this core centre of stygobiont community richness which extends upstream of the gorge for approximately 2 km and downstream for approximately 6 km (Bennelongia 2015) (Fig. 1B). The groundwater quality in the shallow aquifer is predominantly fresh with measured salinities in most

boreholes less than 1500 mg/L but with some boreholes recording salinities up to around 5,000 mg/L (Subterranean Ecology 2014). The highest salinity groundwater that *N. karanovici* sp. n. was collected from was around 2400 mg/L.

The Ethel Gorge aquifer stygobiont community is listed in Western Australia as an endangered Threatened Ecological Community (TEC) by the Department of Parks and Wildlife (DPaW 2014). The Environmental Protection Authority (EPA 2016) has identified potential impacts on stygofauna habitat and species within the Ethel Gorge TEC from mine dewatering groundwater drawdown and changes in water quality due to the discharge of surplus water into Ophthalmia Dam. As a consequence of its proximity to these potential threatening processes the Ethel Gorge aquifer has received greater sustained survey effort (mostly in the form of environmental compliance monitoring) and taxonomic scrutiny (including molecular genetic studies) than any other groundwater system in Australia. For environmental impact assessment (EIA) and environmental compliance monitoring it is pertinent to note that even considering the intensive biannual field sampling and specimen identification efforts over more than 15 years, new species (including *N. karanovici* sp. n.) continue to be detected from boreholes that have been sampled many times previously (Subterranean Ecology 2012, 2013). While this finding is entirely consistent with intensively studied groundwater systems elsewhere in the world (e.g. Culver and Pipan 2009, Dole-Olivier et al. 2015, Brancelj et al. 2016), this facet of subterranean biology is not widely recognised in EIA for mine projects in Australia.

Acknowledgements

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